Environmental Monitoring by Universal PCR

Effective environmental monitoring using a simple assay for measuring and delineating DNA signatures in samples.

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Environmental Monitoring for Target Agents

NIAID Category A & B Priority Pathogens

Category A	Category B
Bacillus anthracis	*Burkholderia pseudomallei*
Clostridium botulinum	*Coxiella burnetti*
Yersinia pestis	*Brucella* species
Francisella tularensis	*Burkholderia mallei*
>16 viruses	*Rickettsia prowazekii*
	Diarrheagenic *E.coli*
	Pathogenic Vibrios
	Shigella species
	Salmonella
	Listeria monocytogenes
	Campylobacter jejuni
	Yersinia enterocolitica
	Cryptosporidium parvum
	Cyclospora cayatanensis
	Giardia lamblia
	Entamoeba histolytica
	Toxoplasma
	Microsporidia
	+ 11 viruses + 3 toxins

One method of environmental monitoring for homeland defense applications is to monitor the levels of specific threat agents in a target environment.

This requires:

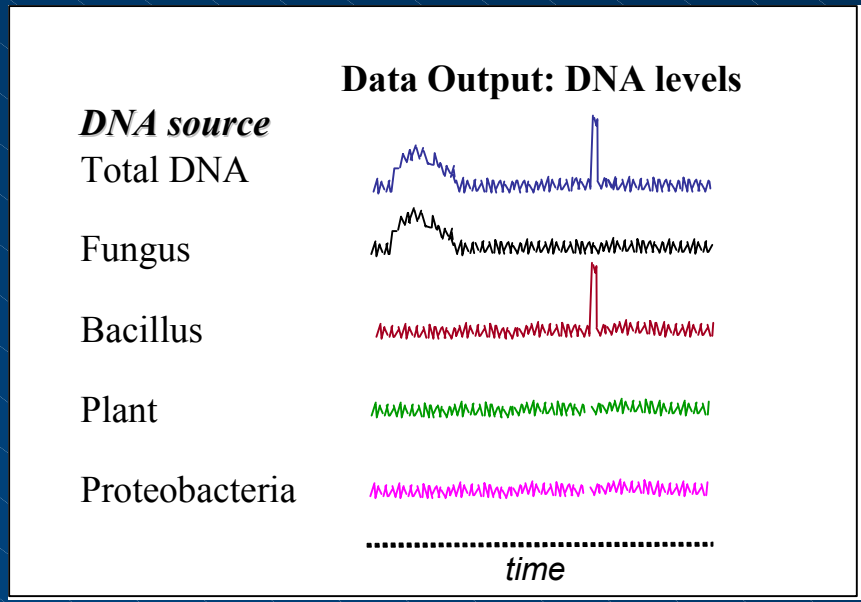
1. **The development and validation of multiple agent-specific assays.**
2. **The application of multiple agent specific assays in each type of environment.**

Environmental Monitoring for Biological Signatures

1. Examine the targeted environment for *multiple environmental signatures* including physical, chemical, biological, and climactic.

2. Analyze data and temporal changes.

3. Develop reliable algorithms and signature processing methods to detect a signal anomaly in the environment.

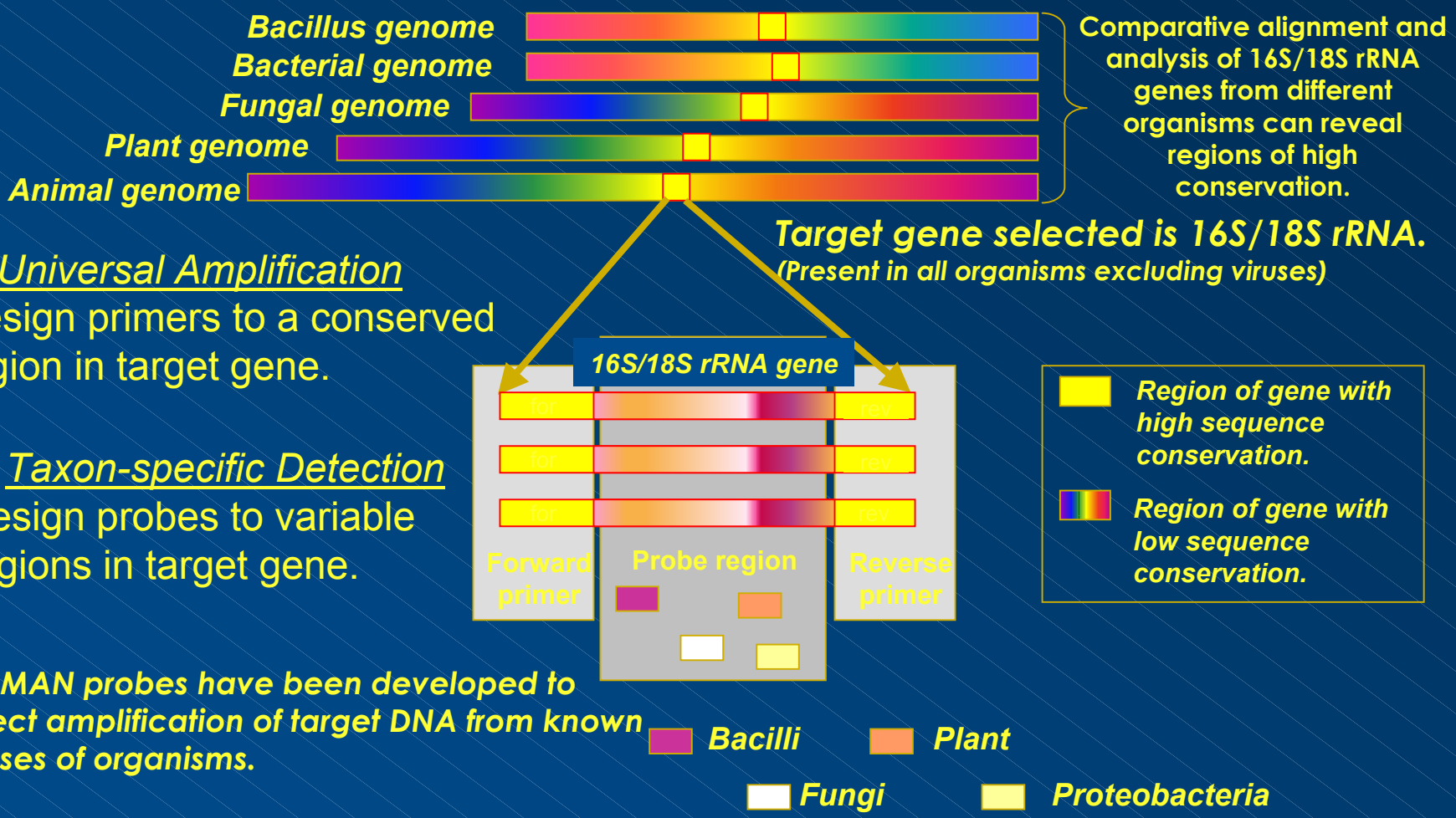


Environmental Monitoring Assay for Biological Signatures

Requirements of Assay for Environmental Monitoring of Biological Signatures

1. Simultaneous analysis of multiple (>10) biological signatures in a sample.
   One assay can be used in multiple types of environments of known and unknown composition.
2. Detection of target and background populations
3. Detection of genetically-altered agents undetectable by agent-specific assays
4. Cost-effective

Universal PCR Methodology



Universal Amplification

Primer Validation

Representative Organisms demonstrating a positive response in a SYBR-Green Universal PCR Reaction

Bacteria-

Firmicutes
Bacteroidetes
Proteobacteria

Archaea

Euryarchaeotoa

Eucarya/Fungi

Ascomycota

Eucarya/Plant

Embryophyta

Eucarya/Animal

Chordata

Taxon-specific Detection

Probe Validation

TAQMAN probes were tested in the PCR assay using DNA with varying target similarity.

Kingdom-level probe

Family-level probe

Class-level probe

Universal PCR Probe Validation

Annex A from Biological Weapons Convention List (2001)
and NIAID Category A and B pathogens

Data not Available

Only 3 probes (=PCR reactions) required to target the taxonomic class of ALL the bacteria.

In addition, this method will also detect genetically-modified variants of each of these.

Reduces time, cost and complexity of analysis.

Environmental Monitoring Data

Environmental monitoring data from air samples in Virginia.

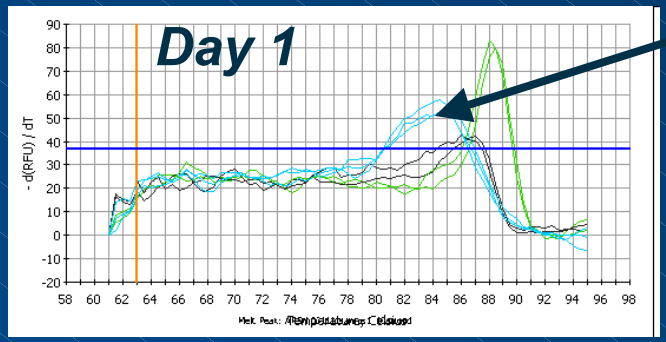
Environmental Monitoring Data

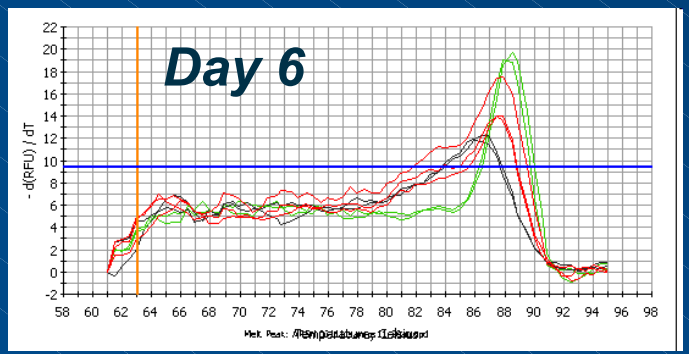
Amplicons from SYBR Green PCR reaction were cloned and sequenced from 5 separate days.

Data not Available

Environmental Monitoring Instrumentation Developments

Representative melt curves from the air-environmental study.





Broad melt curve from an environmental sample suggesting the presence of multiple amplicon signatures.

New instrumentation available that is specifically suited to analyze complex melt curves.

An Environmental Monitoring Assay for Multiple Types of Environments

The molecular fingerprinting approach described here can be applied to any sample containing cellular or DNA material.

However, the value of the retrieved data increases in value as more molecular fingerprints are obtained for a sample or environment.

AIR	WATER	SOIL
Background	Background	Background
Fungi	Algae	Fungi
Plant	Plant	Actinobacteria
Targets	Targets	Targets
Bacillus	Cryptosporidia	Bacillus
Yersinia	Bacillus	*Yersinia*

Environmental Monitoring PCR vs. Agent Monitoring PCR

Environmental Monitoring PCR

- One PCR assay can be used in multiple scenarios.
- Indicates the presence of DNA from potential warfare agents.
- Indicates levels of background DNA.
- Can detect DNA from genetically-modified organisms.
- Valuable as a control in diagnostic PCR.
- PCR Results are evaluated within the context of the sample.
- Provides broad taxonomic data on the content of a sample.
- Method has not been developed for use for virus detection.

Agent Monitoring PCR

- Requires multiple PCR assays to target multiple threat agents.
- Requires continual assay development to target emerging threat agents.
- Provides data on presence or absence of specific threat agent.
- Can detect and identify viral, bacterial and protozoan targets.

Summary

You get what you look for.

Obtain as many characteristic signatures as possible from a sample.

The accumulation of multiple data sources in environmental monitoring can provide a reliable warning of a suspect biological signature.

- Biological data
- Meteorological data
- Chemical data
- Physical data